ASSESSMENT OF MATING STATUS OF FEMALE GRASSHOPPERS[1]

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ABSTRACT: A technique is described whereby the spermatheca and duct are removed from field-caught adult female grasshoppers and stained for spermatozoa, thus enabling the mating status of the individual to be determined.

During studies on reproductive strategies of grasshoppers in areas of California with a Mediterranean climate (Weissman and French, 1979), it was necessary to determine the time of insemination by a technique independent of observing field pairs *in copulo*. An alternative method is especially important in species with low densities where spermatophore transfer might take only 30-40 minutes (see, for example, Pickford and Gillott, 1971; Haskell, 1960) and, consequently, go unobserved. This paper describes such a method, whereby, using testicular spermatozoa for comparison, it was ascertained that females of the oedipod grasshopper *Trimerotropis occidentalis* (Bruner) went unmated for 9-14 weeks after becoming adult as part of their reproductive dormancy strategy (see Weissman and French, 1979, for details).

METHODS

The spermatheca and its duct (see Uvarov, 1966, p. 145 for anatomy) of the female, and the testes of the male are removed in insect saline from recently captured adults and immediately fixed in freshly prepared 3 parts 100% ethanol: 1 part glacial acetic acid. The structures may be examined immediately, or can be stored in fixative in a freezer for years. The spermatheca and duct are blotted dry of fixative, placed on a glass slide, stained with lacto-propionic orcein, macerated with a small rod, protected with a coverslip, and examined at low (125X) power with a compound microscope. Three to five follicles are isolated from a testis and treated in the same manner.

RESULTS AND DISCUSSION

Spermatozoa are easier to detect in the spermatheca after they have been

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initially appreciated, in the absence of other confusing structures, by first examining testicular follicles where *mature* sperm should be readily visible (Fig. 1). Spermathecal spermatozoa will appear identical (Fig. 2), but cellular wall structures (Figs. 3, 4, and 5) can also have a stringy appearance similar to spermatozoan bundles. In unmated females, this distinction between wall components and sperm is both crucial and especially difficult. The final decision with reference to actual insemination should be made only after searching properly squashed spermatheca and finding diagnostic isolated spermatozoon (Fig. 6).

Figure 1. Mature spermatozoa appear as long filaments (arrows) in partial testis squash of *Trimerotropis occidentalis.*

Figure 2. *T. occidentalis* female with spermathecal squash revealing presence of sperm (arrows) morphologically indistinguishable from those seen in Fig. 1.

Figures 3, 4, and 5: Various spermathecal suborgan structures (arrows), most likely of wall origin, from a female *T. occidentalis,* that must be distinguished from spermatozoa. The grasshopper was in reproductive dormancy and unmated at time of capture.

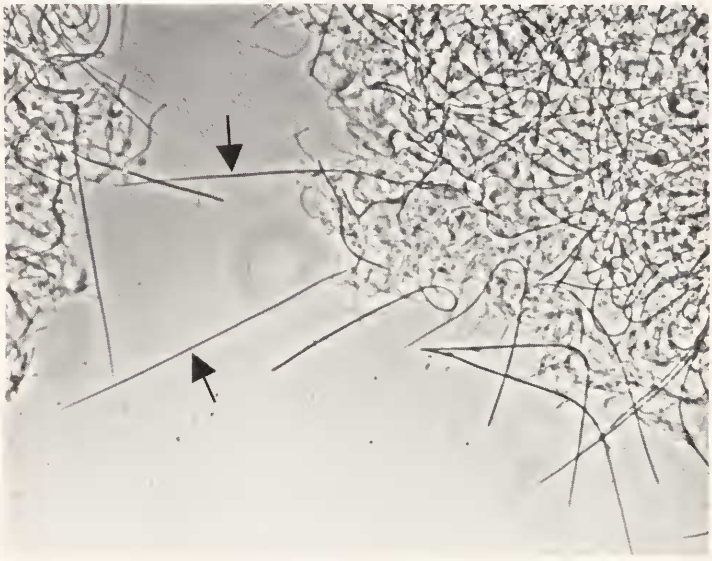

Figure 6: "Single" spermatozoon (arrows, under phase contrast) as seen in *T. occidentalis* spermathecal squash. Similar structures are not seen in virgin females and are diagnostic of insemination.

Using this method, I was also able to confirm the delayed mating in the spur-throated grasshopper *Melanoplus devastator* Scudder, as originally postulated by Middlekauff (1964). This technique should be applicable to all invertebrates possessing a spermatheca, or its functional equivalent.

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